

Project Description¹

iGEM 2022, University of Sheffield

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¹ from <https://2022.igem.wiki/sheffield/description>

Why we chose directed evolution and how we are doing it

What Inspired *rEvolver*?

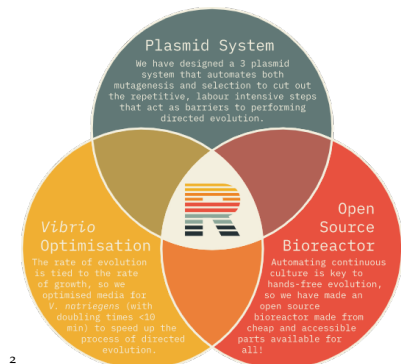
At its heart, synthetic biology is about integrating the biological sciences with engineering principles and design. As our understanding of biology has expanded and matured, it has become possible to apply molecular biology concepts and design novel solutions to problems instead of waiting for them to be discovered in nature. Typically these solutions come in the form of engineered proteins, but our knowledge of their biochemistry and mechanics is often limiting when it comes to rationally designing or enhancing an enzyme's function. There is, however, another way to enhance and adapt these macromolecular machines: directed evolution.

In directed evolution, only a baseline knowledge of a protein's structure and function is required for its optimisation. Instead of needing to understand every inter- and intramolecular reaction and interaction, the scientist needs only to provide a pool of mutated gene sequences and a mechanism for selecting the most fit individuals. While this approach is theoretically much more accessible to smaller labs and iGEM teams, in practice it can be astonishingly labour and time intensive. One round of directed evolution, if that's the only work being done in the lab, can take up to a week, and these experiments often entail a dozen or more rounds of DNA extraction, mutation, transformation, expression, and selection (see our chats with Michael Magaraci on the Human Practices page). It's for these reasons that "discontinuous" directed evolution is often outside the realm of possibility for many.

It was the great promise of directed evolution being locked behind this impracticality that drove our team to action this year. Just as iGEM set out to bring synthetic biology to new scientists from around the globe, we set out to bring directed evolution to the masses.²

How Does *rEvolver* Make Directed Evolution Easy?

The first step in making directed evolution more widely accessible was reducing the amount researcher involvement required in the lab. For this, we turned to an approach called in vivo continuous directed evolution. By replacing laborious steps like DNA extraction,



mutation, and retransformation with in vivo diversity generation mechanisms and coupling that with a continuous selection process, the process can be made almost entirely hands-free. In the past, the field of continuous directed evolution has been dominated by phage-based technologies such as PACE, but these approaches come with all of the complexities and drawbacks of culturing live phage — putting all of the bacteriological work in a lab at risk of phage contamination and lysis.

Unlike phage, plasmids are a safer tool that nearly all molecular biologists are accustomed to working with. Building a continuous in vivo directed evolution system without phage would theoretically retain many of the benefits of previous projects like Heidelberg 2017³ but further lower the barrier to entry for fledgling scientists. Making this all possible is the groundwork in targeted mutagenesis laid by teams like Evry Paris-Saclay 2021⁴ and the work in growth-modulating genes done by teams like Imperial 2016⁵. These parts laid the basis for our Three Plasmid System⁶ — with one plasmid for targeted in vivo mutation using MutaT7, another for continuous selection using a growth-slowing or enhancing gene under the control of a fitness-monitoring biosensor, and a third plasmid for knocking down / out host DNA repair genes (increasing the efficiency of mutation).

While our modular plasmid system removes the need for researchers to continually be manipulating cells and DNA, it doesn't shorten how long these cells take to grow! To make directed evolution even more accessible to scientists short on time, we've optimised all of our genetic parts and protocols for use with *Vibrio natriegens* — an up-and-coming chassis for synthetic biology. With doubling times of under 10 minutes, the time taken by evolution experiments could be halved (according to our evolutionary modelling⁷). Working with *V. natriegens* also gives us an opportunity to build on the excellent work of the Marburg 2018⁸ team which recently published the excellent Marburg collection of *V. natriegens* compatible parts. To further supercharge the growth of our *V. natriegens* cells, we set out on a media optimisation quest⁹ guided by a Design of Experiments approach. With both *V. natriegens* optimised parts and media, rEvolver aims to drive evolution faster than ever before.

Finally, while the accelerated growth of our *V. natriegens* cells makes evolution easy, it makes them a bit tricky to keep fed — particularly for experiments that can last weeks at a time! To save researchers from making tedious daily (and nightly) trips to re-inoculate or dilute cultures, we designed an affordable, easy-to-build bioreactor.¹⁰ With this device and our three-plasmid system, researchers can “transform and forget” — leaving their cells to grow

³ <https://2017.igem.org/Team:Heidelberg>

⁴ https://2021.igem.org/Team:Evry_Paris-Saclay

⁵ https://2016.igem.org/Team:Imperial_College

⁶ <https://2022.igem.wiki/sheffield/three-plasmid-system>

⁷ <https://2022.igem.wiki/sheffield/model>

⁸ <https://2018.igem.org/Team:Marburg>

⁹ <https://2022.igem.wiki/sheffield/media-circus>

¹⁰ <https://2022.igem.wiki/sheffield/hardware>

unattended in a bioreactor for weeks at a time before returning to receive their evolved gene.

Finally, though it forms a fully separate branch of our project, our bioinformatics toolbox¹¹ follows the same ethos as the rest of our project — making science more accessible to all. Our toolbox does this by drawing together common DNA, RNA, and protein sequence manipulation tools from around the web into a single, sleek web interface.

¹¹ <https://2022.igem.wiki/sheffield/software>

References

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